

09/120,044

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DIALOG INFORMATION SERVICES

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Set Items Description

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?b 155

29oct99 11:42:02 User233831 Session D163.1

\$0.32 0.099 DialUnits File1

\$0.32 Estimated cost File1

\$0.01 TYMNET

\$0.33 Estimated cost this search

\$0.33 Estimated total session cost 0.099 DialUnits

File 155:MEDLINE(R) 1966-1999/Dec W3

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Set Items Description

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?s pneumolysin(5n)mutant

159 PNEUMOLYSIN

94542 MUTANT

S1 13 PNEUMOLYSIN(5N)MUTANT

?t s1/3,ab/1-13

1/3,AB/1

DIALOG(R) File 155:MEDLINE(R)

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09997596 99270945

Role of Pneumolysin's complement-activating activity during pneumococcal bacteremia in cirrhotic rats.

Alcantara RB; Preheim LC; Gentry MJ

Veterans Affairs Medical Center, Omaha, Nebraska, USA.

Infect Immun (UNITED STATES) Jun 1999, 67 (6) p2862-6, ISSN 0019-9567
Journal Code: GO7

Languages: ENGLISH

Document type: JOURNAL ARTICLE

We investigated the role of pneumolysin's complement-activating activity during *Streptococcus pneumoniae* bacteremia in a hypocomplementemic, cirrhotic host. Isogenic **mutant** pneumococcal strains, in which **pneumolysin** was expressed from a plasmid, were used. These strains included H+C+, expressing wild-type pneumolysin with both cytolytic and complement-activating activity; PLY-, carrying the plasmid without the pneumolysin gene; and, H+C-, expressing pneumolysin with cytolytic activity only. In control rats, intravenous infection with 2.0×10^7 CFU of H+C+ per ml of blood resulted in a decrease in bacteremia of 3.5 log units by 18 h postinfection and 55% mortality. By contrast, cirrhotic rats infected similarly with the H+C+ strain demonstrated a 0.2-log-unit increase in bacteremia by 18 h postinfection and 100% mortality. Both control and cirrhotic rats cleared the PLY- strain more effectively from their bloodstreams by 18 h postinfection (6.2 and 5.6 log unit decreases, respectively). Infection with the PLY- strain also resulted in low mortality (0 and 14%, respectively) for control and cirrhotic rats. When infected with the H+C- strain (without complement-activating activity), both groups cleared the organism from their bloodstreams nearly as well as they did the PLY- strain. Furthermore, the mortality rate for control and cirrhotic rats was identical after infection with the H+C- strain. These studies suggest that pneumolysin production contributes to decreased pneumococcal clearance from the bloodstream and higher mortality in both control and cirrhotic rats. However, pneumolysin's complement-activating activity may uniquely enhance pneumococcal virulence in the hypocomplementemic, cirrhotic host.

1/3,AB/2

DIALOG(R) File 155:MEDLINE(R)

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09861537 99113007

Pneumolysin in pneumococcal adherence and colonization.

Rubins JB; Paddock AH; Charboneau D; Berry AM; Paton JC; Janoff EN

Department of Medicine, Veterans Affairs Medical Center and University of Minnesota School of Medicine, Minneapolis, MN, 55417, USA.

Microb Pathog (ENGLAND) Dec 1998, 25 (6) p337-42, ISSN 0882-4010
Journal Code: MIC

Contract/Grant No.: AI-042240, AI, NIAID; AI-39445, AI, NIAID; HL-57880, HL, NHLBI

Languages: ENGLISH

Document type: JOURNAL ARTICLE

The universal and highly conserved production of pneumolysin, the major pneumococcal cytotoxin, among clinical isolates of *Streptococcus pneumoniae* and the previously reported association of pneumolysin production with increased pneumococcal adherence to respiratory epithelium in organ cultures suggest that this toxin might be important for nasopharyngeal colonization. We confirmed that **pneumolysin**-deficient **mutant** pneumococcal strains had decreased adherence to respiratory epithelial cells in vitro compared with their isogenic wild-type strains. However, neither early nor sustained colonization by type 14 *S. pneumoniae* in an established murine model was dependent on bacterial production of

pneumolysin. We conclude that pneumolysin production is not a major determinant of successful nasopharyngeal colonization by pneumococci.
Copyright 1998 Academic Press

1/3,AB/3

DIALOG(R) File 155:MEDLINE(R)

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09859595 99115586

Comparative virulence of Streptococcus pneumoniae strains with insertion-duplication, point, and deletion mutations in the pneumolysin gene.

Berry AM; Ogunniyi AD; Miller DC; Paton JC

Molecular Microbiology Unit, Women's and Children's Hospital, North Adelaide, S.A., 5006, Australia.

Infect Immun (UNITED STATES) Feb 1999, 67 (2) p981-5, ISSN 0019-9567
Journal Code: G07

Languages: ENGLISH

Document type: JOURNAL ARTICLE

Pneumolysin is a 471-amino-acid toxin produced by Streptococcus pneumoniae which has both cytolytic and complement activation properties. We have constructed a derivative of the type 2 S. pneumoniae strain D39 in which the portion of the pneumolysin gene encoding amino acids 55 to 437 has been deleted in-frame. The virulence of this strain (DeltaPly) was compared with those of wild-type D39, a **pneumolysin** insertion-duplication **mutant** (PLN-A), and a derivative (PdT) carrying a toxin gene with three point mutations known to abolish both cytolytic activity and complement activation. PdT was intermediate in virulence between D39 and either PLN-A or DeltaPly in a mouse intraperitoneal challenge model. This provides unequivocal evidence that pneumolysin has an additional property that is not abolished by point mutations which reduce cytotoxicity and complement activation to virtually undetectable levels.

1/3,AB/4

DIALOG(R) File 155:MEDLINE(R)

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09768643 98451771

Multivalent pneumococcal capsular polysaccharide conjugate vaccines employing genetically detoxified pneumolysin as a carrier protein.

Michon F; Fusco PC; Minetti CA; Laude-Sharp M; Uitz C; Huang CH; D'Ambra AJ; Moore S; Remeta DP; Heron I; Blake MS

North American Vaccine, Inc., Beltsville, Maryland, USA. fmichon@nava.com

Vaccine (ENGLAND) Nov 1998, 16 (18) p1732-41, ISSN 0264-410X
Journal Code: X60

Languages: ENGLISH

Document type: JOURNAL ARTICLE

A genetically detoxified pneumolysin, pneumolysoid (PLD), was investigated as a carrier protein for pneumococcal capsular polysaccharide (CPS). Such a CPS-PLD conjugate might provide additional protection against pneumococcal infections and resultant tissue damage. A single point **mutant** of **pneumolysin** was selected, which lacked measurable haemolytic activity, but exhibited the overall structural and immunological properties of the wild type. PLD conjugates were prepared from CPS serotypes 6B, 14, 19F, and 23F by reductive amination. The structural features of free PLD, as well as the corresponding CPS-PLD, as assessed by circular dichroism spectroscopy, were virtually indistinguishable from the wild type counterpart. Each of the CPS monovalent and tetravalent conjugate formulations were examined for immunogenicity in mice at both 0.5 and 2.0 micrograms CPS per dose. Tetanus toxoid (TT) conjugates were similarly created and used for comparison. The resultant conjugate vaccines elicited high levels of CPS-specific IgG that was opsonophagocytic for all serotypes tested. Opsonophagocytic titres, expressed as reciprocal dilutions resulting in 50% killing using HL-60 cells, ranged from 100 to 30,000, depending on the serotype and formulation. In general, the lower dose and

tetravalent formulations yielded the best responses for all serotypes (i.e., either equivalent or better than the higher dose and monovalent formulations). The PLD conjugates were also generally equivalent to or better in CPS-specific responses than the TT conjugates. In particular, both the PLD conjugate and the tetravalent formulations induced responses for type 23F CPS that were approximately an order of magnitude greater than that of the corresponding TT conjugate and monovalent formulations. In addition, all the PLD conjugates elicited high levels of pneumolysin-specific IgG which were shown to neutralize pneumolysin-induced haemolytic activity in vitro. As a result of these findings, PLD appears to provide an advantageous alternative to conventional carrier proteins for pneumococcal multivalent CPS conjugate vaccines.

1/3,AB/5

DIALOG(R)File 155:MEDLINE(R)

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09505932 98181051

Amino acid changes affecting the activity of pneumolysin alter the behaviour of pneumococci in pneumonia.

Alexander JE; Berry AM; Paton JC; Rubins JB; Andrew PW; Mitchell TJ

Department of Microbiology and Immunology, University of Leicester, Leicester, LE1 9HN, U.K.

Microb Pathog (ENGLAND) Mar 1998, 24 (3) p167-74, ISSN 0882-4010

Journal Code: MIC

Contract/Grant No.: AI34051, AI, NIAID

Languages: ENGLISH

Document type: JOURNAL ARTICLE

Pneumolysin is a multi-functional toxin produced by *Streptococcus pneumoniae*. The toxin has distinct cytotoxic activity and complement-activating activity mediated by different parts of the toxin molecule. Mice challenged intranasally with a type 2 pneumococcal strain contract bronchopneumonia and bacteremia [1]. Mice were infected intranasally with isogenic mutants of this strain in which the chromosomal pneumolysin gene carried point mutations affecting either or both properties of pneumolysin. Reduction in either cytotoxic activity or complement activation by **pneumolysin** decreased the virulence of the **mutant** pneumococci. However, it was the ability to activate complement that most affected the behaviour of pneumococci in the lungs and associated bacteremia in the first 24 h following infection. Copyright 1998 Academic Press Limited.

1/3,AB/6

DIALOG(R)File 155:MEDLINE(R)

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09388857 98114397

Role of tumor necrosis factor alpha in the host response of mice to bacteremia caused by pneumolysin-deficient *Streptococcus pneumoniae*.

Benton KA; VanCott JL; Briles DE

Department of Microbiology, The University of Alabama at Birmingham, 35294, USA.

Infect Immun (UNITED STATES) Feb 1998, 66 (2) p839-42, ISSN 0019-9567

Journal Code: GO7

Contract/Grant No.: AI21548, AI, NIAID; AI07051, AI, NIAID

Languages: ENGLISH

Document type: JOURNAL ARTICLE

Pneumolysin-deficient **mutant** strains of *Streptococcus pneumoniae* are known to cause less-severe sepsis than wild-type pneumococcal strains that produce pneumolysin. This difference is associated with greater host resistance in mice infected with the pneumolysin-deficient strains. These studies show that the host resistance developed during the first 1 to 2 days after infection with a **pneumolysin**-deficient **mutant** strain is dependent on tumor necrosis factor alpha but is apparently independent of interleukin 1beta (IL-1beta) or IL-6. Survival beyond 5 days appeared to

depend on the ability of the mice to produce IL-1beta.

1/3,AB/7

DIALOG(R) File 155:MEDLINE(R)

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09020033 97230293

Differences in virulence for mice among Streptococcus pneumoniae strains of capsular types 2, 3, 4, 5, and 6 are not attributable to differences in pneumolysin production.

Benton KA; Paton JC; Briles DE

Department of Microbiology, The University of Alabama at Birmingham, USA.
kbenton@uab.edu

Infect Immun (UNITED STATES) Apr 1997, 65 (4) p1237-44, ISSN 0019-9567 Journal Code: GO7

Contract/Grant No.: AI21548, AI, NIAID; AI07051, AI, NIAID

Languages: ENGLISH

Document type: JOURNAL ARTICLE

We observed that differences in the in vivo growth kinetics of pneumococcal strains of capsular types 3, 4, 5, and 6 were reminiscent of differences that we had previously reported for type 2 strain D39 and its **pneumolysin** -deficient **mutant**, PLN. Capsular type 2 Streptococcus pneumoniae D39 exhibits exponential growth in the blood of XID mice until the death of the mice at 24 to 36 h. In contrast, PLN reaches a plateau in growth that is maintained for several days. Capsular type 3 and 5 strains exhibited exponential growth and caused rapid death of XID mice following intravenous challenge, similar to the observation with D39. Strains of capsular types 4 and 6 exhibited growth kinetics reminiscent of PLN. Since the observed differences in the pathogenesis of types 3 and 5 compared to 4 and 6 were reminiscent of the effects of pneumolysin deficiency in type 2, we examined the levels of in vitro pneumolysin production for the entire panel of strains. The onset of pneumolysin production in most strains was rapid and occurred near the end of log-phase growth. Differences in in vivo growth patterns of capsular type 2, 3, 4, 5, and 6 strains were not found to be associated with differences in the levels of pneumolysin.

1/3,AB/8

DIALOG(R) File 155:MEDLINE(R)

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08399622 95386973

The limited role of pneumolysin in the pathogenesis of pneumococcal meningitis.

Friedland IR; Paris MM; Hickey S; Shelton S; Olsen K; Paton JC; McCracken GH

Department of Pediatrics, University of Texas Southwestern Medical Center, Dallas, USA.

J Infect Dis (UNITED STATES) Sep 1995, 172 (3) p805-9, ISSN 0022-1899 Journal Code: IH3

Languages: ENGLISH

Document type: JOURNAL ARTICLE

The aim of this study was to determine the role of pneumolysin, an intracellular toxin of Streptococcus pneumoniae, in the pathogenesis of pneumococcal meningitis. Recombinant pneumolysin (1 microgram), when injected intracisternally into rabbits, resulted in a brisk inflammatory response. However, a pneumolysin-deficient strain of S. pneumoniae caused meningeal inflammation in rabbits indistinguishable from that induced by the parent pneumolysin-producing strain. Furthermore, similar enhancement of meningeal inflammation occurred after ampicillin therapy in animals infected with either the parent strain or the **pneumolysin** -deficient **mutant**. These results suggest that although **pneumolysin** can stimulate the inflammatory cascade in the central nervous system, it is not necessary for the pathogenesis of meningeal inflammation nor does it play a role in postantibiotic enhancement of meningeal inflammation.

1/3,AB/9

DIALOG(R) File 155:MEDLINE(R)

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08359004 95330957

Growth and virulence of a complement-activation-negative mutant of *Streptococcus pneumoniae* in the rabbit cornea.

Johnson MK; Callegan MC; Engel LS; O'Callaghan RJ; Hill JM; Hobden JA; Boulnois GJ; Andrew PW; Mitchell TJ

Department of Microbiology and Immunology, Tulane University School of Medicine, New Orleans, LA 70112, USA.

Curr Eye Res (ENGLAND) Apr 1995, 14 (4) p281-4, ISSN 0271-3683

Journal Code: DUB

Contract/Grant No.: EY00424, EY, NEI; EY02377, EY, NEI; EY08871, EY, NEI

Languages: ENGLISH

Document type: JOURNAL ARTICLE

Our previous work has demonstrated the importance of pneumolysin in the virulence of *S. pneumoniae* in a rabbit intracorneal model. This was accomplished by showing that deletion of the gene encoding pneumolysin resulted in reduced virulence, whereas restoration of the wild-type gene resulted in restoration of the virulent phenotype. To assess the importance of a particular domain in the pneumolysin molecule, we have now constructed a strain which produces a pneumolysin molecule which is hemolytic but which bears a site-specific mutation in the domain known to be associated with the complement-activating properties of this molecule. Comparison of the virulence of this strain with that of a strain bearing the wild-type gene showed statistically significantly lower total slit lamp examination (SLE) scores at 12, 18, 24, and 36 h (particularly with respect to fibrin formation), but no difference at 48 h. Determination of colony forming units (CFU) in eyes infected with the two strains showed approximately 10(6) bacteria per cornea until 36 h. Between 36 and 48 h, the bacteria were almost completely cleared with very few bacteria recoverable at the later time point. The loss of virulence observed with this mutation in the complement-activation domain of pneumolysin, though less than that observed with the gene deletion mutant, suggests that complement activation by pneumolysin has a significant role in the pathology observed in this model of corneal infection.

1/3,AB/10

DIALOG(R) File 155:MEDLINE(R)

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08258196 95122173

A pneumolysin -negative mutant of *Streptococcus pneumoniae* causes chronic bacteremia rather than acute sepsis in mice.

Benton KA; Everson MP; Briles DE

Department of Microbiology, University of Alabama at Birmingham.

Infect Immun (UNITED STATES) Feb 1995, 63 (2) p448-55, ISSN 0019-9567

Journal Code: GO7

Contract/Grant No.: AI21548, AI, NIAID

Languages: ENGLISH

Document type: JOURNAL ARTICLE

Pneumolysin is a cytoplasmic virulence factor of *Streptococcus pneumoniae* that can interfere with phagocyte function in vitro. We have examined the effects of pneumolysin in vitro and in vivo and have found that it protects intravenously injected pneumococci against infection-induced host resistance. We employed a virulent capsular type 2 pneumococcal strain, D39, and its isogenic pneumolysin -negative mutant, PLN. Strain D39 exhibited exponential net growth in mice (doubling time, 1.4 h); 24 to 28 h after infection with 10(4) CFU, the numbers of pneumococci reached 10(9) to 10(10) CFU/ml and the mice died. Strain PLN yielded identical net growth in mice until reaching 10(6) to 10(7) CFU/ml at 12 to 18 h postinfection. At this time, the increase in the level of PLN CFU per milliliter ceased and remained constant for several days. PLN exhibited wild-type growth kinetics in mice when coinfecting simultaneously with strain D39. This observation

suggests that pneumolysin exerts its effects at a distance. By 12 to 18 h postinfection with PLN, mice exhibited the following evidence of an induced inflammatory response: (i) elevated plasma interleukin-6, (ii) a halt in the net growth of PLN, and (iii) control of the net growth of pneumolysin-producing D39 pneumococci upon subsequent challenge. Our data suggest that pneumolysin plays a critical role in sepsis during the first few hours after infection by enabling pneumococci to cause acute sepsis rather than a chronic bacteremia. However, once chronic bacteremia was established, it appeared that pneumolysin was no longer able to act as a virulence factor.

1/3,AB/11

DIALOG(R) File 155:MEDLINE(R)

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07980875 94345500

Characterisation of an oxidative response inhibitor produced by *Streptococcus pneumoniae*.

Perry FE; Elson CJ; Mitchell TJ; Andrew PW; Catterall JR

Department of Pathology and Microbiology, University of Bristol.

Thorax (ENGLAND) Jul 1994, 49 (7) p676-83, ISSN 0040-6376

Journal Code: VQW

Languages: ENGLISH

Document type: JOURNAL ARTICLE

BACKGROUND--Pneumonia caused by infection with *Streptococcus pneumoniae* is still a major clinical problem. Reactive oxygen species contribute to the killing of these bacteria by polymorphonuclear leucocytes (PMNs). Defence mechanisms of *Str pneumoniae* which counter reactive oxygen species are characterised. METHODS--PMNs were stimulated with phorbol myristate acetate (PMA) in the presence and absence of *Str pneumoniae* and supernatants from them, and superoxide (O₂⁻) production was measured by the reduction of ferricytochrome c. RESULTS--*Streptococcus pneumoniae*, but not *Klebsiella pneumoniae* or *Staphylococcus aureus*, inhibited PMA stimulated superoxide production by PMNs. Washed PMNs which had been preincubated with *Str pneumoniae* autolysis phase supernatants also exhibited depressed H₂O₂ production in response to PMA. The inhibitory activity was not attributable to non-specific cytotoxicity as assessed by release of the cytoplasmic enzyme lactate dehydrogenase, nor did the supernatants inhibit PMA stimulated degranulation of PMNs. Fractionation of the autolysis phase supernatants revealed inhibitory activity in both the fractions greater than and less than 10 kD. Like pneumolysin the inhibitory activity was heat sensitive. However, both a parent and **pneumolysin negative mutant** *Str pneumoniae*, and autolysis phase supernatants from them, inhibited PMN superoxide production. Antisera to pneumolysin failed to abrogate the inhibitory effect of intact *Str pneumoniae* or autolysis phase supernatants from types 1 or 14 *Str pneumoniae*. CONCLUSIONS--The inhibitory effect of *Str pneumoniae* on the respiratory burst of PMNs is not shared by two other common lung pathogens. The existence of a novel inhibitor of the PMN respiratory burst, distinct from pneumolysin, has been demonstrated. The inhibitor is specific for the respiratory burst and is active both in the logarithmic phase of growth and during autolysis.

1/3,AB/12

DIALOG(R) File 155:MEDLINE(R)

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07843781 94011332

Identification of hydrogen peroxide as a *Streptococcus pneumoniae* toxin for rat alveolar epithelial cells.

Duane PG; Rubins JB; Weisel HR; Janoff EN

Department of Medicine, Minneapolis Veterans Affairs Medical Center, Minnesota.

Infect Immun (UNITED STATES) Oct 1993, 61 (10) p4392-7, ISSN 0019-9567 Journal Code: GO7

Contract/Grant No.: AI31373, AI, NIAID; R29-AI34051, AI, NIAID

Languages: ENGLISH

Document type: JOURNAL ARTICLE

Streptococcus pneumoniae infections of the lung are associated with significant damage to the alveolar epithelium. Host phagocytes and pneumolysin, a cytolytic toxin of *S. pneumoniae*, are believed to contribute to this cellular damage, yet experiments in which these elements are absent demonstrate the presence of an additional soluble *S. pneumoniae* factor that is toxic to alveolar epithelium. We examined the effects of *S. pneumoniae*-associated alveolar epithelial cell injury by factors other than *S. pneumoniae*-derived pneumolysin or phagocyte products by exposing cultured rat type II alveolar epithelial cells (RAEC) to *S. pneumoniae* mutants that lacked **pneumolysin** activity. We found that **mutant pneumolysin**-deficient strains of *S. pneumoniae* produced injury to RAEC similar to that produced by the parent strains. A toxin of type 14 *S. pneumoniae* was distinguished from pneumolysin by physiochemical (i.e., molecular mass and heat stability) and functional (i.e., hemolytic activity and cytotoxic activity) properties and was identified as hydrogen peroxide. All *S. pneumoniae* strains tested produced hydrogen peroxide, and in many strains hydrogen peroxide production was comparable to that of activated neutrophils. We conclude that *S. pneumoniae* produces hydrogen peroxide in concentrations that are cytotoxic to RAEC in vitro and that alveolar epithelial damage due to hydrogen peroxide may be involved in the pathogenesis of host cellular injury in pneumococcal pneumonia.

1/3,AB/13

DIALOG(R) File 155:MEDLINE(R)

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05567646 89277519

Reduced virulence of a defined pneumolysin-negative mutant of *Streptococcus pneumoniae*.

Berry AM; Yother J; Briles DE; Hansman D; Paton JC

Department of Microbiology, Adelaide Children's Hospital, Australia.

Infect Immun (UNITED STATES) Jul 1989, 57 (7) p2037-42, ISSN 0019-9567 Journal Code: GO7

Contract/Grant No.: T32H007300; AI8557, AI, NIAID; AI21548, AI, NIAID

Languages: ENGLISH

Document type: JOURNAL ARTICLE

Insertion-duplication mutagenesis was used to construct a pneumolysin-negative derivative of *Streptococcus pneumoniae*. This was achieved by first transforming the nonencapsulated strain Rx1 with a derivative of the vector pVA891 carrying a 690-base-pair DNA fragment from the middle of the pneumolysin structural gene. DNA was extracted from the resultant erythromycin-resistant, pneumolysin-negative rough pneumococcus and used to transform *S. pneumoniae* D39, a virulent type 2 strain. Several erythromycin-resistant transformants were obtained from two independent experiments, and none of these produced pneumolysin. Southern blot analysis confirmed that the pneumolysin gene in these transformants had been interrupted by the plasmid-derived sequences. The pneumolysin-negative mutants showed reduced virulence for mice compared with D39, as judged by survival time after intranasal challenge, intraperitoneal 50% lethal dose, and blood clearance studies. Pneumolysin production was reinstated in one of the mutants by transformation with the cloned pneumolysin gene, with the concomitant loss of erythromycin resistance; the virulence in mice of this isolate was indistinguishable from that of D39. These results confirm the involvement of pneumolysin in pneumococcal pathogenesis.